BOSTON UNIVERSITY

GRADUATE SCHOOL

Thesis

THE MICRO METHOD IN ORGANIC CHEMISTRY

by

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P 7505

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INTRODUCTION

In an effort to save time and materials, and to develop an additional technique in the laboratory, the micro method has been adapted to a course in general organic chemistry. In doing this, small-size apparatus and reduced amounts of materials (one cubic centimeter and less of liquids and one-tenth of a gram and less of solids) were used. Some reactions were carried out under the microscope. The centrifuge was used for settling precipitates. This method was adapted to some of the laboratory processes such as distillation, filtration and the washing of precipitates. Finally, in order to test the micro method, a group of experiments which have been used in general organic chemistry were performed. The results were very satisfactory. Such a group of experiments will form the basis of a micro general organic chemistry course.

The author wishes to extend her thanks to Mr. Gorden McKey and Mr. John Hopkins, assistants in the Biology and Chemistry departments respectively for their assistance in the taking of the micro-photographs of crystals.

INTRODUCTION

VALUES AND ADVANTAGES OF THE MICRO METHOD

1. Economy of Time. As a result more experiments may be covered in a given amount of time or by decreasing the number of laboratory hours the cost of the course may be lessened.

2. Economy of Space. Since the amount of supplies is less, the stock room space is also decreased. For the strictly micro course, there is no need for the large apparatus and space required for the macro organic course. Students are able to do most of their work sitting down at their desks. This reduces the noise and rushing around in the laboratory.

3. Economy of Materials. The very much smaller amounts of materials called for in the experiments results in less materials being required for the whole class.

In the macro course there is the tendency for students to use more reagent than the test requires but in the micro method the directions must be followed explicitly or the test may be lost. This eliminates the wasting of materials.

The use of such minute amounts of materials makes possible a class study of the rarer compounds and the more expensive reagents of organic chemistry which is impossible in the macro course.

4. Safety. With the use of such small amounts of materials odors are eliminated or greatly reduced, and the hazards of fire, explosions and poisoning are negligible. This makes a more healthy laboratory for the students.

5. Technique. In macro chemistry it is often diffi-

cult to get the student to keep his apparatus clean. This is very essential in micro work, for here a speck of impurity is a much larger percentage in a single drop on a slide than it is in a beaker of water. Also traces of grease on a slide make difficult such operations such as micro decantation and filtration.

Since in the micro method the directions must be followed very closely in order to get the desired test, a much greater accuracy is developed in the student.

The student learns something about the microscope and its principles, crystals, microscopical photography. In this way organic chemistry becomes much broader in mature.

6. Accuracy. The method is just as accurate as the macro method and in many cases more so.

7. Expense. The initial cost of the micro course is low and its upkeep is much lower than that of the macro course.

LITERATURE

The literature in the field of micro methods in chemistry is quite extensive, but most of it deals with inorganic chemistry, especially qualitative analysis. Two authors, Emich and Pregl however, deal with organic micro methods. Since many of the laboratory processes are common to both organic as well as inorganic chemistry, some helpful information has been gathered from the literature. The references are included in the bibliography.

APPARATUS

Much of the glass apparatus used in micro organic chemistry such as pipettes, stirrers, melting point tubes and micro distillation apparatus can be made by the students themselves, by drawing out glass tubing or rodding and sealing.

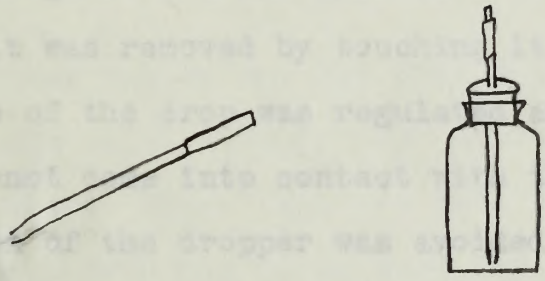
Pipettes were drawn from 6 mm. glass tubing. The best method found to make these was by heating a small area of the glass tubing across a wing top burner and then pulling. In this way a pipette was obtained which had a rapidly tapering end. If the end tapers gradually, there is great danger of its breaking. The bore of the pipette was cut such that it released about 20 drops to one cubic centimeter. The final length of the pipette was 10 centimeters.

The liquid was drawn into the pipette by allowing it to rise by capillary attraction, then by holding the pipette between the thumb and middle finger and placing the index finger over the end of the pipette, the liquid was let out by releasing the index finger. In order to use capillary attraction there must be a column of liquid in the bottle nearly equal in height to that desired in the pipette.

A better method was found. The end of the pipette was fitted with a piece of rubber tubing, 3 to 4 centimeters in length. Then by placing the index finger over the end of the tubing and by pinching the rubber tubing with the thumb and the middle finger, the liquid was suctioned into the pipette; and by releasing the index finger the liquid flowed out drop by

drop. It is necessary that the student have many pipettes, because after a pipette has been used for one solution it should not touch another until it has been cleaned.

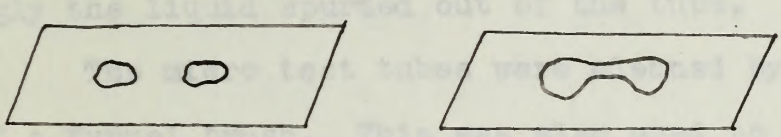
Reagent bottles should be fitted with these pipettes or droppers. When the pipette was used in the bottle as a dropper, it was found to be more effective if a glass plug was placed in the end of the rubber tubing on the pipette, as shown in the diagram below. In this way the reagents remain clean from dust in the air and also from impurities which might be introduced into them. The bore of the droppers or pipettes were also calibrated so that 20 drops equalled one cubic centimeter.



Each student can be profitably provided with his own set of the common organic reagents such as alcohol, ether, chloroform, benzene, acetone and so forth as well as the common inorganic acids and bases. These should be in dropping bottles because this will save the student time and avoid contamination of his reagents. If each student has his own set of reagents the congestion about the stock or supply shelf will be relieved.

Stirring rods were made by drawing out glass rodding and then firepolishing the ends. They were used for mixing

drops on a slide as shown in the diagram below. Each drop was placed on the slide near to one another. Then they were mixed by drawing one into the other. In this way zones of different concentrations were present.



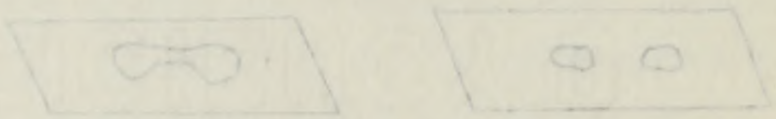
Stirring rods were also used as sampling rods for transfering drops of liquids from pipettes to the glass slides. The pipette containing the desired liquid was removed from the dropping bottle and a drop of the liquid was placed about half way up on the glass rod and allowed to slide down to the end from which it was removed by touching it to the slide. In this way the size of the drop was regulated and the point of the pipette did not come into contact with the test drop. Thus contamination of the dropper was avoided.

It was found that if care was taken in using the pipette of the dropping bottle, it need not come into contact with the test drop. The pipette was brought close to the slide and the drop allowed to fall on the slide just to the side of the test drop. This method was found to be the better after a little practice and took the least time in the end.

If a test tube was desired that was smaller than the 2" ones, they were made by sealing a short length of glass tubing.

Forceps were bent at the ends of their prongs to form

drops on a slide as shown in the diagram below. Each drop was placed on the slide near to one another. Then they were mixed by drawing one into the other. In this way series of different concentrations were present.



Stirring rods were also used as sampling rods for transferring drops of liquids from pipettes to the glass slides. The pipette containing the desired liquid was removed from the dropping bottle and a drop of the liquid was pulled about half way up on the glass rod and allowed to slide down to the end from which it was removed by drawing it to the slide. In this way the size of the drop was regulated and the point of the pipette did not come into contact with the test drop. Thus contamination of the dropper was avoided.

It was found that if care was taken in using the pipette of the dropping bottle, it need not come into contact with the test drop. The pipette was brought close to the slide and the drop allowed to fall on the slide just to the side of the test drop. This method was found to be the better after a little practice and took the least time in the end.

If a test tube was desired that was smaller than the 5" ones, they were made by heating a short length of glass tubing.

Forceps were bent at the ends of rubber prongs to form

a micro test tube holder. In most cases the test tubes could be held in the hand when placed in the flame to heat the contents of the tube. The tube could be kept there long enough to heat but yet not burn the hand. If the tube was heated too strongly the liquid spurted out of the tube.

The micro test tubes were cleaned by using the small end of a funnel brush. This was also used to clean the ends of the centrifuge tubes.

Pipettes were cleaned by running water from the tap through them.

A micro test tube rack was made by punching holes in the cover of a shallow box, and standing the test tubes in the holes.

Rubber tubing was cut into one centimeter lengths to serve as one holed stoppers for micro test tubes.

A micro water bath was made by using a 30 or 50 cc. beaker. If the bath was to be used for test tubes only the 30 cc. beaker was used. If a 30 cc. beaker was to be heated on the bath the 50 cc. beaker was used.

The spot plate was found to be very convenient for mixing solutions, diluting solutions and for color tests.

Glass slides were also used for color tests because the slide could be placed against different backgrounds, white, black or colored in order to show up the color to its best advantage. Its special use was for crystal formation. The slide may then be placed immediately under the microscope and

the crystals viewed on the same slide that they were formed on.

Micro burners were available but they were very hot and heated only in one spot. For this reason a certain technique was necessary in using them so that the apparatus being heated did not crack or break. However there were several good substitutes. A candle on a block of wood was very efficient. A Bunsen burner was used if it was turned down very low and placed not too near the object being heated. A blow pipe was arranged by attaching a rubber tubing to the gas jet as usual and then clamping the blow pipe rod to a ring stand. These all gave a small and yet not too hot a flame.

Ideally each student should have his own microscope and centrifuge, but one microscope and centrifuge can be used by a group of students without much difficulty.

On the next page is included a tentative list of the apparatus necessary for each student doing micro organic chemistry. These pieces were used in the performing of the experiments found further on in this thesis.

LIST OF APPARATUS

2" test tubes

4" test tubes

smaller tubes drawn from glass tubing

forceps for test tube holder

box for test tube rack

rubber tubing for stoppers and pipettes

centrifuge tubes

funnel brush

glass rod stirrers

pipettes

microscope slides

spot plate

black glazed paper for background

filter paper, ordinary and hardened

suction filter

Hirsch funnel

porcelain crucible, size 0

beakers, 30 and 50 cc.

watch glasses, 1 and 3"

reagent bottles

burners: Bunsen, micro, blow pipe, candle

distilling flasks, 25 cc.

funnel, 100 cm.

Microscope, 1 for each group

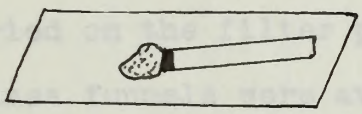
centrifuge, 1 for each group

LABORATORY PROCESSES

Filtration.

There are several methods of filtration which have been used successfully. Each one serves a different purpose.

To separate a liquid from a solid on a slide, a piece of soft filter paper about the size of standard litmus was used to absorb the liquid from the mixture by holding the filter paper at the edge of the drop. Since the liquid was lost in this method on the filter paper, it was most satisfactory only where the solid was desired.



The centrifuge is another device used in filtration to separate the precipitate from the liquid. A small hand centrifuge with four compartments was used. Each compartment contained an aluminum holder for a 15 cc. centrifuge tube. After the mixture had been centrifuged the supernatant liquid was decanted or suctioned off with a pipette. The residue was left in the tip of the tube where it was packed very firmly. The residue was washed in the same tube by adding the washing material, stirring the mixture and then centrifuging again. The supernatant liquid or washing liquid was then decanted or suctioned off again. This process was carried out as many times in the same tube as there were necessary washings, and with as many different kinds of washing liquids as necessary. This

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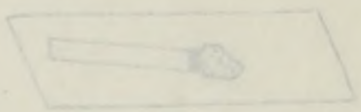

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method worked well where the liquid was desired and when a very fine precipitate had to be thrown down. It could be used for all filtrations if so desired.

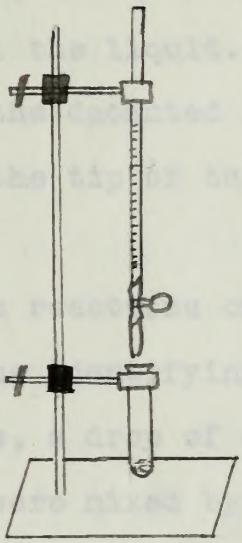
If either the residue or the filtrate was desired, or both, and especially if the reaction was carried out in a test tube or a beaker, a Hirsch funnel was used, either with suction or in the ordinary method. Suction was applied by setting the funnel in a rubber stopper in either a small suction flask or in a test tube with a side arm which was then attached to the suction pump. The size of the filter paper used on this apparatus was about the size of a nickel. In this type of filtration the precipitate was dried on the filter paper if so desired.

Micro glass funnels were available which were used in the ordinary manner or under suction like the Hirsch funnels described above.

Titration.

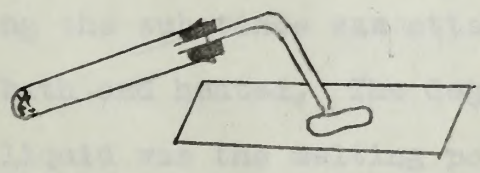
Titration on the micro scale was carried out in 1 or 2 cc. pipettes which were graduated to tenths of a cc. These were suspended in a rubber stopper which was placed in a clamp on a ring stand. A glass dropper attached to a piece of rubber tubing carrying a pinch clamp was placed at the lower end of the pipette. This attachment was worked like those on the full size burettes. When the apparatus was thus arranged, the liquid was sucked up into the pipette. Then the pinch clamp was closed, and the height of the liquid in the pipette recorded. The tip of the glass dropper was wiped off before beginning

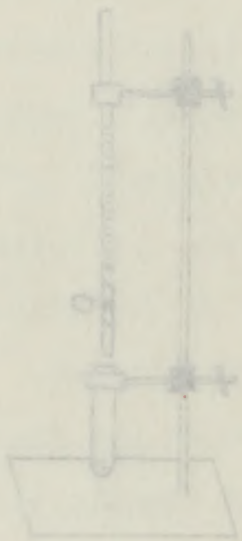
the actual titration to prevent any extra unrecorded liquid from dripping into the solution from the micro burette and thus causing an error. The apparatus was then ready for the actual titration which was carried out in the same manner as the macro titration.



Gas generation.

A small test tube fitted with glass tubing which is bent and drawn to a fine point was used as the container for the gas generating materials. A small piece of rubber tubing served as the stopper between the test tube and the bent tube. The glass tubing was then led into the solution or test drop where the gas was needed. When such small quantities were used, the amount of air to be replaced had to be reduced to a minimum, and in this method the 2" test tube worked very well.





Decantation.

If the precipitate was not too fine and if the separation did not have to be too perfect, the solid was allowed to settle, and the separation or decantation effected in the usual way. Otherwise, it was found efficient to centrifuge first and then decant the liquid. In this method no precipitate came over with the decanted liquid since the solid was lodged so firmly in the tip of the tube.

Crystallization.

Many of the reactions on a micro scale involve the use of crystals as the identifying criteria. To form crystals directly on the slide, a drop of each solution was placed on the slide and these were mixed by means of a glass stirrer. The mixing consisted of drawing one drop into the other so that there were areas of different concentrations. The reaction and formation of crystals was then watched under the microscope and the concentration which was the most favorable could be determined.

Microphotographs were taken of some of the best and more beautiful crystals for permanent record. (See experiments)

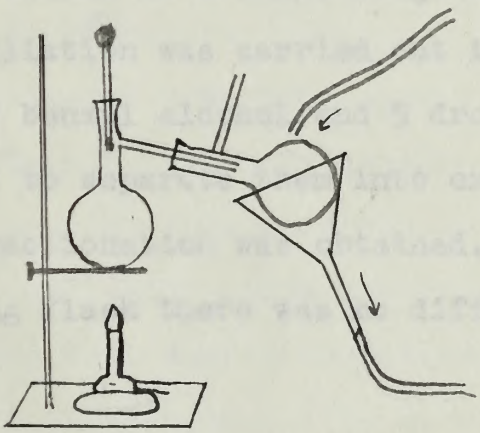
Melting Points.

The determination of melting points was exactly the same as that in the macro course. A capillary tube sealed at one end containing the substance was attached to a thermometer in a 50 cc. oil bath and heated. The degree at which the substance became a liquid was the melting point.

Distillation.

One method was used to distill quantities of about 5 cc. with very good success. This was the use of a 25 cc. distilling flask the side arm of which led into the mouth of a similar flask the side arm of which remained open. The bulb of the second flask was placed in a large funnel. A steady flow of cold water was allowed to pass over the bulb and through the funnel thus cooling the gases in the bulb. The stem of the funnel was connected by rubber tubing to the sink.

A freezing mixture was added to the funnel to reduce the temperature and so condense the gases. The heat could not be applied too rapidly to the first flask because then the gases were generated faster than the condensing apparatus could take care of them and so some of the gas vapor passed out the open side arm and was lost.

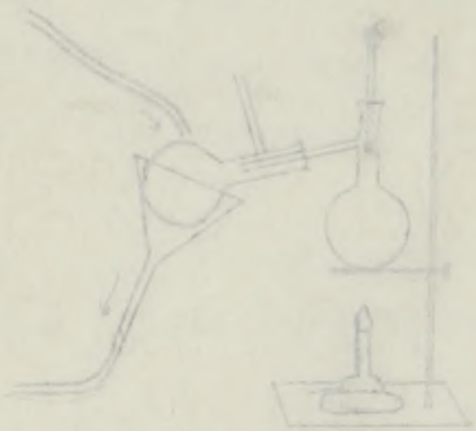


A micro distilling flask blown by Mr. Everett Kilmer was used to distill quantities of 10 drops. To fill this flask a pipette was used. This had to be inserted way down into the bulb of the flask otherwise some of the liquid remained on the shelf and went over into the distillate without being distilled.

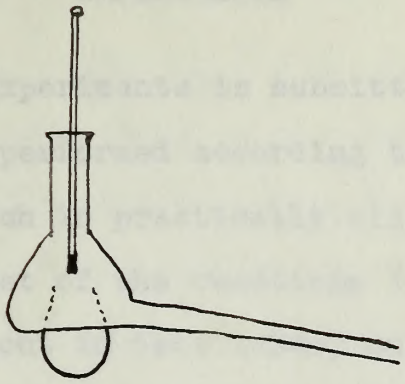
Distillation.

One method was used to distill quantities of about 5 cc. with very good success. This was the use of a 25 cc. distilling flask the side arm of which led into the mouth of a smaller flask the side arm of which remained open. The bulb of the second flask was placed in a large funnel. A steady flow of cold water was allowed to pass over the bulb and through the funnel thus cooling the gases in the bulb. The stem of the funnel was connected by rubber tubing to the sink.

A freezing mixture was added to the funnel to reduce the temperature and so condense the gases. The heat could not be applied too rapidly to the first flask because then the gases were generated faster than the condensing apparatus could take care of them and no some of the gas vapor passed out the open side arm and was lost.



A micro distilling flask blown by Mr. Everett Hilger was used to distill quantities of 10 drops. To fill this flask a pipette was used. This had to be inserted way down into the bulb of the flask otherwise some of the liquid remained on the shelf and went over into the distillate without being distilled.



The heat had to be applied gently otherwise the liquid boiled over into the distillate. The distillate was collected in a micro test tube. A thermometer was inserted in the top of the apparatus but when an ordinary one was used it did not indicate true boiling points because of lag; that is by the time the mercury had time to rise up to the point of the true boiling point of the liquid, all the substance had been distilled over. Therefore the thermometer should be a short range one.

Fractional distillation was carried out in this apparatus using 5 drops of benzyl alcohol and 5 drops of ethyl alcohol. It was difficult to separate them into exact halves, but a certain amount of fractionation was obtained. However, using the 25 cc. distilling flask there was no difficulty in getting fractions.

EXPERIMENTS

A group of experiments is submitted in the following pages which have been performed according to the micro method, and the results of which in practically all cases have been very satisfactory. Most of the reactions in the experiments submitted are carried out in test tubes, on slides or on a spot plate. Color reactions in particular are well performed on a slide or a spot plate.

Many of the directions for these experiments have been taken from "Experiments in Organic Chemistry" by Lyman C. Newell, Ph.D., of Boston University, which are for the macro method. Others were suggested by J. Philip Mason, Ph.D. also of Boston University. It is hoped that by working over this group of experiments, a good micro organic program can be obtained for either one or two semesters.

Some of the experiments have been performed by Dr. Mason's class in General Organic Chemistry according to the micro method, namely, the Quantitative Determination of Glucose by Benedict's Method, and Amino Acids and Proteins. The results of these experiments were very satisfactory and consistent with the results of those done on the macro scale.

PROPERTIES OF AMYLENE

1. One cc. water was added to 2 drops of amylene in a micro test tube. The mixture was shaken. The amylene was insoluble in water and the specific gravity was less than one because the amylene rose to the top of the solution. This test did not work well on a white spot plate because both solutions were colorless, thus no color contrast. The test was difficult to see even on a black background. On a slide or a spot plate the specific gravity cannot be determined.

2. Six drops of a 2% solution of bromine in carbon tetrachloride were added to 2 drops of amylene in a clean dry micro test tube. The solution was decolorized quickly.

The same reaction was carried out on a spot plate using 1 drop of amylene and 3 drops of a 2% solution of bromine in carbon tetrachloride. The bromine solution was decolorized immediately.

3. Six drops of an alkaline solution of potassium permanganate (0.1% containing 5 grams of NaOH per liter) were added to 2 drops of amylene in a micro test tube. The mixture was shaken. The color of the solution turned from purple to green and then to brown.

This reaction was carried out on a spot plate using 1 drop of amylene and 3 drops of the alkaline potassium permanganate solution. The color changes were as above. They showed up very well against the white background of the spot plate.

4. Six drops of 10% NaOH solution were added to 2

PROPERTIES OF XYLENE

1. One cc. water was added to 2 drops of xylene in a micro test tube. The mixture was shaken. The xylene was insoluble in water and the specific gravity was less than one because the xylene rose to the top of the solution. This test did not work well on a white spot plate because both solutions were colorless, thus no color contrast. The test was difficult to see even on a black background. On a slide or a spot plate the specific gravity cannot be determined.

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This reaction was carried out on a spot plate using 1 drop of xylene and 3 drops of the alkaline potassium permanganate solution. The color changes were as above. They showed up very well against the white background of the spot plate.

4. Six drops of 10% NaOH solution were added to 2

drops of amylene in a micro test tube. The mixture was shaken. Two layers were formed, no heat was liberated, and there was no evidence of any reaction. This test was repeated on a slide and spot plate but it worked best in the test tube where it was not so difficult to see the two layers as on the slide or spot plate.

5. Six drops of concentrated sulfuric acid were added to 2 drops of amylene in a micro test tube. The mixture was shaken very carefully. A great amount of heat was evolved and the amylene solution became charred. This reaction can also be performed on a slide but the heat effect is not evident.

PROPERTIES OF SATURATED HYDROCARBONS AND GASOLINE

A. Properties of Ligroin.

1. Six drops of 2% solution of bromine in carbon tetrachloride were added to 3 drops ligroin (purified by shaking with potassium permanganate) in a clean dry micro test tube. The test tube was stoppered and allowed to stand on the top of the desk, after the mixture had been shaken. The color of the solution turned from red orange to yellow. After standing a half hour the solution became decolorized. A control was used of 6 drops of 2% solution of bromine in carbon tetrachloride added to 3 drops of carbon tetrachloride. This was shaken, and stoppered and allowed to stand on the top of the desk along with the reaction mixture. The control remained the same color during the entire experiment, therefore the definite change in color in the reaction mixture was due to a chemical chamge.

2. Six drops of an alkaline solution of potassium permanganate (0.1% containing 5 grams of NaOH per liter) were added to 3 drops of ligroin in a micro test tube. The mixture was shaken for one minute. The solution was decolorized from purple to green then to brown. The time for reaction was about 5 minutes.

This reaction was also carried out on a spot plate using 2 drops of ligroin and 3 drops of potaasium permanganate. The reaction was very slow, taking about 15 minutes to get the color change.

3. Six drops of 10% solution of sodium hydroxide were

added to 3 drops of ligroin in a micro test tube and the mixture was shaken. The ligroin remained on top and there was no sign of reaction.

This reaction was performed by the drop method on a slide or on a white spot plate. However, it was difficult to see if the drops mixed and almost impossible to determine which was the sodium hydroxide layer.

4. Six drops of concentrated sulfuric acid were added to 3 drops of purified ligroin and the mixture shaken carefully. The solution became turbid but there was no evidence of heat or of charring.

5. One cc. of water was added to 3 drops of ligroin in a micro test tube and the mixture was shaken. Ligroin was not soluble in water.

6. The testing of the solubility of ligroin in ether, ethanol and acetone was performed by using 3 drops of ligroin to 1 cc. of the solvent. Ligroin was soluble in all three.

B. Properties of Paraffin Oil.

1. Six drops of a 2% solution of bromine in carbon tetrachloride were added to 3 drops of paraffin oil in a clean dry micro test tube. The mixture was shaken and the test tube stoppered and left standing for a day when the solution became decolorized. A control was used of 6 drops of a 2% solution of bromine in carbon tetrachloride added to 3 drops of carbon tetrachloride. This test tube was stoppered and allowed to stand with the reaction mixture. There was no change in color.

2. Six drops of an alkaline solution of potassium permanganate (0.1% containing 5 grams of NaOH per liter) were added to 3 drops of paraffin oil in a micro test tube and the mixture was shaken. There was no change in the solution.

3. Six drops of 10% sodium hydroxide solution were added to 3 drops of paraffin oil in a micro test tube. The mixture was shaken and a cloudy emulsion formed. On standing the oil settled to the bottom.

4. Six drops of concentrated sulfuric acid were added to 3 drops of paraffin oil in a micro test tube and the mixture was shaken carefully. After 5 minutes the solution became charred.

5. One cc. of water was added to 3 drops of paraffin oil in a micro test tube and the mixture was shaken. Paraffin oil is not soluble in water and its specific gravity is less than one.

6. The testing of the solubility of paraffin oil in ether, ethanol and acetone was performed by using 3 drops of paraffin oil and 1 cc. of solvent. Paraffin oil was soluble in ether. It forms a cloudy emulsion with alcohol which on standing changes, the oil rising to the top. It is insoluble in acetone and rises to the top of the solution.

C. Properties of Gasoline.

1. Six drops of 2% solution of bromine in carbon tetrachloride were added to 3 drops of gasoline in a clean dry micro test tube. The solution was decolorized immediately.

2. Six drops of an alkaline solution of potassium permanganate (0.1% containing 5 grams of sodium hydroxide per liter) were added to 3 drops of gasoline in a micro test tube. The mixture was shaken for one minute. The solution changed color from a purple to a dark brown.

3. Six drops of 10% sodium hydroxide solution were added to 3 drops of gasoline in a micro test tube and the mixture was shaken. The liquids did not mix, the gasoline remaining on the top of the solution.

4. Six drops of concentrated sulfuric acid were added to 3 drops of gasoline in a micro test tube and the mixture was shaken. In 2-3 minutes the solution became charred.

5. One cc. of water was added to 3 drops of gasoline in a micro test tube and the mixture was shaken. The gasoline was not soluble in water and its specific gravity was less than one.

6. The testing of the solubility of gasoline in ether, ethanol and acetone was performed by using 3 drops of gasoline and 1 cc. of the solvent. Gasoline was soluble in all three.

DETECTION OF ETHANOL

PREPARATION OF IODOFORM

Method A. Two drops of 10% NaOH solution were added to 4 drops of water and an equal amount of ethanol which had been mixed in a test tube. To this mixture, 2 drops of 10% solution of iodine in potassium iodide were added. The odor of iodoform was detected and a yellow precipitate of iodoform crystals was formed. Some of these crystals were placed on a slide and were viewed under the microscope. They were compared with those shown on page 79 in Hawk and Bergeim's "Practical Physiological Chemistry," They were found to be typical and very beautiful.

Method B. After many attempts using different quantities, this procedure was found to be the best. On a slide, 1 drop of 10% NaOH solution, 2 drops of water, 2 drops of ethanol and 1 or 2 drops of 10% solution of iodine in potassium iodide were mixed. The yellow of iodoform and its characteristic odor were detected. These crystals were observed under the microscope directly on the slide upon which they were formed. A photomicrograph is shown on the next page.

Solution. The 10% iodine solution in potassium iodide was made as follows: a saturated solution of potassium iodide was made by dissolving 20 grams of potassium iodide in 100 cc. of water. To 5 cc. of this solution 1 gram of iodine was added. When solution had taken place, it was diluted with 5 cc. of water.

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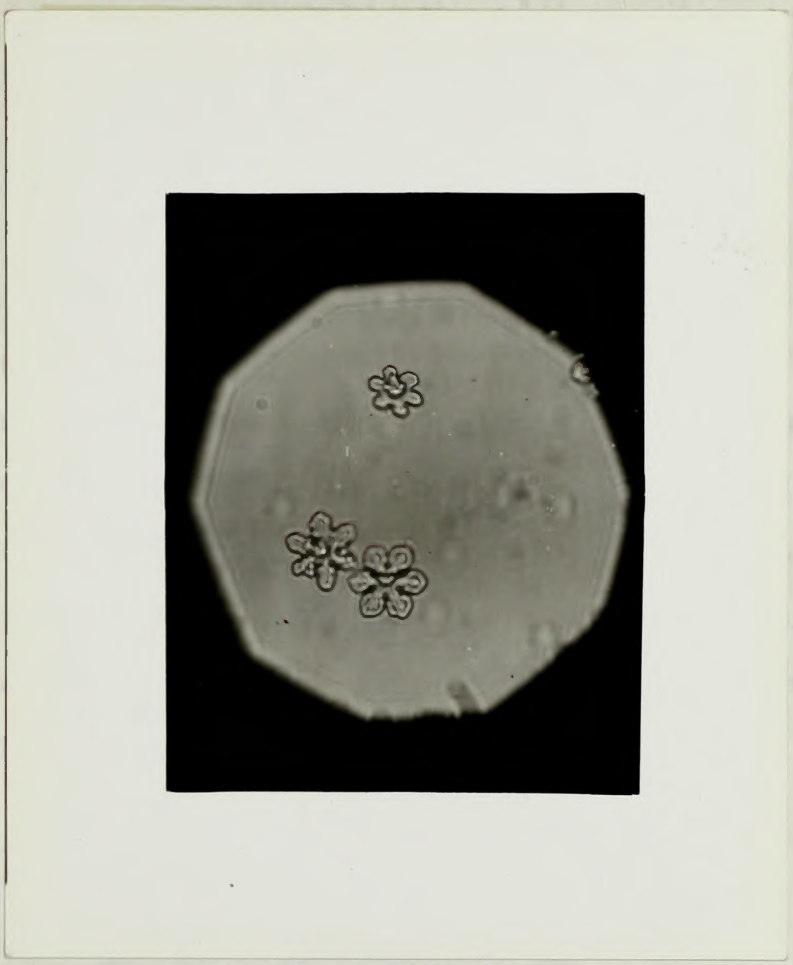
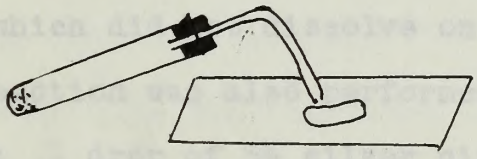


Iodoform (x 385)

Iodoform (x 385)

PROPERTIES OF OXALIC ACID

1. Reaction with sulfuric acid. A micro test tube was fitted with a bent glass tube drawn to a fine bore and a rubber connector serving as a stopper, as shown in the diagram. Three drops of 5% oxalic acid solution and 5 drops of concentrated sulfuric acid were carefully heated in the test tube. The escaping gas was ignited. It burned with a blue flame proving that CO was given off. Then the gas was poured into another test tube containing 10 drops of saturated $Ba(OH)_2$ solution which became cloudy showing that CO_2 was present. The gas was also led into a test drop of saturated $Ba(OH)_2$ solution on a slide, instead of using the test tube as above. The drop became cloudy therefore carbon dioxide.



2. Formation of calcium oxalate. A slight excess of NH_4OH (4-6 drops) was added to 3 drops of 5% solution of oxalic acid in a micro test tube. To this, 3 drops of 10% calcium chloride solution were added. A white precipitate of calcium oxalate was formed. The mixture was divided into two parts. To one part, 7 drops of dilute acetic acid were added. The precipitate was insoluble. To the other part, 7 drops of

dilute hydrochloric acid were added. The precipitate dissolved.

This reaction was also carried out on a microscope slide using the following quantities: 1 drop of 5% oxalic acid solution, 2 drops of ammonium hydroxide and 1 drop of calcium chloride. A white precipitate of calcium oxalate was formed. The drop containing the precipitate was divided using a glass stirring rod into 2 equal parts on the slide. To one part, 3 drops of dilute acetic acid were added. The precipitate did not dissolve. To the other part, 3 drops of dilute hydrochloric acid were added. The precipitate dissolved. The precipitate of calcium oxalate when viewed under the microscope gave very fine crystals with no specific shape.

3. Formation of silver oxalate. Two drops of 5% silver nitrate were added to 2 drops of 5% oxalic acid solution in a micro test tube. A white precipitate of silver oxalate was formed which did not dissolve on heating.

This reaction was also performed on a microscope slide as follows: 1 drop of 5% silver nitrate solution was added to 1 drop of 5% oxalic acid solution. A white precipitate was formed. This did not dissolve when the slide was held over the flame for a minute. The precipitate when viewed under the microscope had no specific shape.

4. Reducing action of oxalic acid. (a) One drop of dilute sulfuric acid and 3 drops of 2% potassium permanganate solution were added to 5 drops of 5% oxalic acid solution or to an oxalate in a micro test tube. The solution turned from

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4. Reducing action of oxalic acid. (a) One drop of dilute sulfuric acid and 3 drops of 5% potassium permanganate solution were added to 5 drops of 5% oxalic acid solution or to an oxalate in a micro test tube. The solution turned from

purple to a faint yellow in a minute.

The same reaction was carried out on a spot plate using the following quantities: 4 drops of oxalic acid, 1 drop of dilute sulfuric acid, and 3 drops of 2% potassium permanganate solution. The time taken to get the color change was a little longer than in the test tube reaction above.

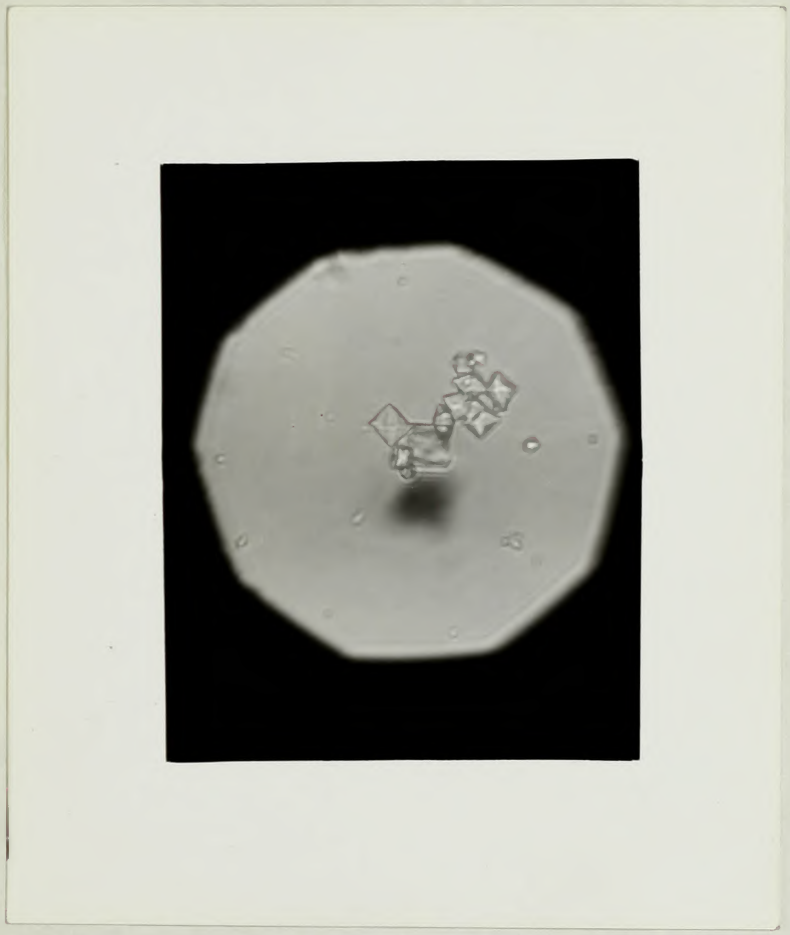
(b) One drop of dilute sulfuric acid and 3 drops of potassium dichromate solution were added to 5 drops of 5% oxalic acid solution or an oxalate in a micro test tube. The solution turned from yellow to purple in a minute.

The same reaction was carried out on a spot plate using the following quantities: 4 drops of oxalic acid, 1 drop of dilute sulfuric acid and 3 drops of potassium dichromate solution. The time for the solution to turn from yellow to purple was a little more than a minute. Therefore this method is slower than the test tube method.

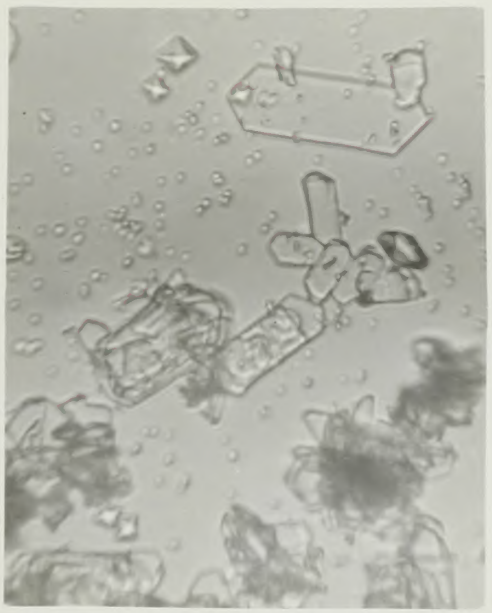
5. Formation of strontium oxalate. One drop of 5% oxalic acid solution and 1 drop of 10% strontium nitrate solution were placed on a microscope slide. The two drops were mixed. The crystals which formed were viewed under the microscope and compared with those shown on page 150 in Lindsley's "Industrial Microscopy." These crystals corresponded to those shown in a neutral solution.

One drop of 10% strontium nitrate solution was mixed on a slide with 1 drop of dilute acetic acid. Then 1 drop of 5% oxalic acid was added to this mixture. Crystals formed

which corresponded to those shown in an acid medium. Microphotographs are shown below of these crystals in the two different mediums.



Strontium Oxalate--neutral medium (x 385)



Strontium Oxalate--acid medium (x 385)

PROPERTIES OF LACTIC ACID

1. Behaviour on heating. Ten drops of 85% lactic acid were heated in a micro test tube. Gas and water were given off and an odor of burnt sugar was detected.

2. Test for an alpha hydroxy acid. Four drops of water were mixed with 1 drop of 0.1% ferric chloride on a white spot plate. The solution remained practically colorless. In another portion of the plate 4 drops of 0.1% lactic acid were mixed with 1 drop of 0.1% ferric chloride. This solution became a deep yellow which is a positive test for an alpha hydroxy acid.

3. Lactic acid and sulfuric acid. Three drops of 85% lactic acid and 5 drops of concentrated sulfuric acid were heated in a micro test tube fitted with a bent glass tubing drawn out to a fine bore and a rubber connector serving as a stopper. The gas that was given off was ignited at the end of the glass tubing. It burned with a blue flame showing that CO was present. Then the gas was poured into another micro test tube containing a saturated $Ba(OH)_2$ solution. This did not become cloudy which showed the absence of CO_2. The gas was also poured into a drop of saturated $Ba(OH)_2$ on a slide and here again there was no reaction.

4. Reducing action of lactic acid. One drop of sulfuric acid and 3 drops of 2% potassium permanganate solution were added to 5 drops of 10% lactic acid in a micro test tube. The color changed from purple to yellow showing the reducing

action of lactic acid.

The same reaction was carried out on a spot plate using the following quantities: 4 drops of 10% lactic acid, 1 drop of dilute sulfuric acid and 3 drops of 2% potassium permanganate solution. The color change was the same as in the test tube reaction but on the spot plate the reaction was a little slower.

One drop of dilute sulfuric acid and 3 drops of 2% potassium dichromate solution were added to 5 drops of 10% lactic acid in a micro test tube. The color of the solution changed from yellow to purple almost immediately.

This reaction was also carried out on a spot plate using the following quantities: 4 drops of 10% lactic acid, 1 drop of dilute sulfuric acid and 3 drops of 2% potassium dichromate solution. The color change was the same as in the test tube reaction but on the spot plate the reaction was a little slower.

5. Uffleman's Reaction. To make Uffleman's reagent, 1 drop of $FeCl_3$ (10%) was added to 10 drops of 2% phenol. One drop of 10% lactic acid by adding it to 9 drops of water was diluted to 10 drops and therefore to a 1% solution. One drop of this 1% solution was by adding it to 9 drops of water again diluted to 10 drops and therefore to a 0.1% solution. One drop of dilute HCl was diluted to 10 drops by adding it to 9 drops of water. In different sections of a spot plate 3 drops of Uffleman's reagent were added to each of the above

dilutions, 1% and 0.1% lactic acid and the diluted HCl. The HCl mixture remained colorless, the 1% lactic acid became yellow and the 0.1% lactic acid became a greenish yellow almost the same as the 1%.

This reaction was also tried with 1% solutions of acetic acid, oxalic acid and malonic acid using the same directions as given above. A very pale yellow was obtained with the oxalic acid but there was no color reaction with either malonic or acetic acids.

PROPERTIES OF TARTARIC ACID AND TARTRATES

1. Behaviour on heating. A speck of tartaric acid was heated in a micro test tube. It became charred and gave off an odor of burnt sugar and some moisture.

This was repeated using a speck of Rochelle Salts. This gave off more moisture, a slight odor of burnt sugar and left a white and viscous liquid.

2. Test for an alpha hydroxy acid. Four drops of water were added to 1 drop of 0.1% $FeCl_3$ solution.on a white spot plate. The 0.1% ferric chloride solution was made by diluting 1 drop of 10% solution to 10 drops, i.e. adding 1 drop ferric chloride solution to 9 drops of water; and then diluting one drop of this solution again to 10 drops, by adding 1 drop of the diluted ferric chloride to 9 drops of water. This very dilute ferric chloride solution remained almost colorless. One drop of 0.1% ferric chloride solution was added to 4 drops of 0.1% tartaric acid solution in another portion of the spot plate. The solution became yellow. This is a positive test for an alpha hydroxy acid.

3. Test for tartrates. One drop of 10% tartaric acid solution was placed on a microscope slide. This was neutralized by 1 drop of ammonium hydroxide. To this 1 drop of 10% calcium chloride solution was added. A white precipitate of calcium tartrate was formed on the slide. The water was drawn off by a small piece of filter paper placed at the edge of the drop of liquid. The precipitate was then dissolved by

the addition of 2 drops of 10% NaOH solution. The liquid on the slide was heated over a small flame and the calcium tartrate was reprecipitated. The crystals were examined under the microscope and were found to be very clearly prismatic pyramids. A microphotograph of these calcium tartrate crystals is shown below.

Calcium Tartrate (x 87)

4. Reducing Action of Tartrates. A micro test tube was cleaned by boiling with 10% NaOH, and then washed with water. In this tube were mixed 3 drops of 5% Rochelle salt solution and 3 drops of ammonium hydroxide. To these were added

2 drops of 2% silver nitrate solution. The test tube containing the mixture was then heated in a hot water bath. In 20 minutes a positive mirror test was obtained. A ring formed on the side of the test tube at the top of the liquid in 15-18 minutes and about 3 minutes later the entire test tube became coated with silver. When the silver nitrate solution was added before the ammonium hydroxide, a half hour passed before a silver mirror was obtained.

QUALITATIVE ANALYSIS OF A MIXTURE OF TARTARIC, OXALIC AND CITRIC ACIDS

The test for an alpha hydroxy acid was first performed. One drop of 0.1% ferric chloride solution was mixed with 4 drops of a solution containing oxalic, tartaric and citric acids on a white spot plate. The mixture became yellow showing the presence of organic acids.

The solution was tested with blue litmus paper, and it was acid. The solution was made neutral with 2 drops of ammonium hydroxide. To ½ cc. of the neutral solution, calcium chloride solution (10%) was added drop by drop until precipitation was complete. This was done in a centrifuge tube. The mixture was then centrifuged until the precipitate had been thrown down. The supernatant liquid was removed by suction with a micro pipette, and was treated under B. This supernatant liquid contained calcium citrate.

A. The residue in the centrifuge tube was boiled with a ½ cc. of dilute acetic acid, and again centrifuged. The supernatant liquid was removed and treated under (b). The residue was again boiled with a ½ cc. of dilute acetic acid and again centrifuged. The supernatant liquid was removed and treated under (b) with that supernatant liquid which was first removed.

(a) The residue was mainly calcium oxalate. This was suspended in 5 drops of dilute sulfuric acid and warmed. This mixture was divided into two equal parts. To the first, 2 drops of dilute potassium permanganate solution were added.

The decolorization of the solution indicated the presence of one of the organic acids, tartaric or oxalic.

To the other part, 2 drops of silver nitrate were added and the mixture neutralized with ammonia. The solution was boiled. A white precipitate was formed showing the presence of oxalic acid.

(b) Part of the solution (3 drops) was evaporated on a microscope slide. A small portion of the salt formed was warmed with 2 drops of concentrated sulfuric acid and 1 drop of pyrogallol. The mixture first became purple and then brown. This indicated the presence of tartaric acid. This was confirmed by the silver mirror test. A micro test tube was cleaned by boiling with NaOH and then washing with water. Two or 3 drops from (b) were placed in the tube and 3 drops of ammonium hydroxide were added. These were mixed by shaking. To this mixture 1 drop of 5% silver nitrate solution was added. The tube was heated in a hot water bath and after 20 minutes there was a silvery deposit which confirmed the presence of tartaric acid.

B. To the supernatant liquid from the first centrifuging a few more drops of calcium chloride were added and the solution boiled for a few minutes. A very slight precipitate formed showing the presence of citric acid. The solution was centrifuged, the supernatant liquid removed and concentrated sulfuric acid added to the residue. The mixture was warmed, charring took place slowly and thus confirmed the presence of citric acid.

CARBOHYDRATES

Molisch Test. One drop of alpha naphthol solution was added to 5 drops of a 2% solution of the carbohydrate. The test tube was inclined and 3 drops of concentrated sulfuric acid were poured down the side of the test tube so that the two layers did not mix. A red ring formed at the zone of contact, which immediately changed to purple. This was a positive test. The solution was shaken and allowed to stand. Then 5 drops of cold water were added and a dull purple precipitate was formed. Then excess ammonium hydroxide was added and the precipitate changed to a yellow-rusty brown. A positive test was obtained with 2% solutions of fructose and sucrose.

Fehling's Test. Two drops of Benedict-Fehling's solution were added to 5 drops of a 1% solution of the carbohydrate and the mixture heated to boiling. A bright red or orange-red precipitate was a positive test. This reaction was also performed on a slide using 2 drops of a 1% solution and 1 drop of Benedict-Fehling's solution. The slide was heated over a micro flame. A bright red or orange-red precipitate was a positive test. Fructose gave a positive test and sucrose gave a negative one.

Aniline Acetate Paper Test. The aniline acetate paper was made by soaking a piece of filter paper (1 x $\frac{1}{4}$ inches) in a beaker containing 5 drops of aniline, 5 drops of glacial acetic acid and 5 drops of water.

A speck of solid carbohydrate was dissolved in 5

drops of hydrochloric acid which had been diluted 1:3 and the mixture was boiled for one minute. The aniline acetate paper was inserted in the test tube containing the mixture and it was boiled for one minute longer. The test paper became pink, which was a positive test. Fructose and sucrose both gave positive tests.

Seliwanoff's Test. Five drops of Seliwanoff's reagent were added to 5 drops of a 2% solution of the carbohydrate in a micro test tube. The mixture was placed in a beaker of boiling water for 20 minutes. A red precipitate formed which was a positive test. Fructose and sucrose both gave positive tests.

Fermentation Test. A shaving of yeast was added to 8 cc. of a 2% solution of the carbohydrate. A micro test tube was filled with this mixture and inverted in a resevoir of the mixture in a 30 cc. beaker. This apparatus was set in a warm place. Within 2 hours gas had collected. This was a positive test. KOH solution was added to the test tube and the mixture shaken. The gas dissolved in the KOH, therefore the gas was carbon dioxide. Maltose gave a positive fermentation test.

Barford's Test. Five drops of Barford's solution were heated to boiling. Five drops of a 2% solution of the

carbohydrate were added to this, heating after the addition of each drop. A red precipitate was formed which was a positive test.

This reaction was also performed on a microscope slide using 1 drop of Barford's solution and heating the slide over the micro flame. Then 1 drop of the carbohydrate solution was added and the mixture heated. A red precipitate was formed which was a positive test. Fructose gave a positive test but sucrose gave a negative one.

Hydrolysis Test. One drop of concentrated hydrochloric acid was added to 5 drops of the starch solution and the mixture boiled for 5 minutes. The solution was neutralized with NaOH using litmus as an indicator. Then Fehling's test was applied and positive result was obtained. This test is applied only when the Fehling's test gives a negative result. The hydrolysis converted the starch into glucose which gives a positive Fehling's test.

Iodine Test. Two drops of iodine solution (very dilute) were added to 5 drops of a 2% starch solution in a micro test tube. A characteristic blue color was obtained which was a positive test.

Phenylhydrazine Test. One drop of phenylhydrazine and 2 drops of glacial acetic acid were added to 10 drops of 2% solution of carbohydrate. The mixture was shaken and suspended in a boiling water bath, using a 30 cc. beaker for this purpose. Crystals formed in a certain amount of time depending

upon the carbohydrate contained in the original solution. (See table below) The crystals were allowed to remain in the bath one minute after forming. Then they were placed on a slide and examined under the microscope. Microphotographs were taken of these various osazone crystals. (See Below)

TIME TABLE OF OSAZONE FORMATION

Arabinose	10-15 minutes
Fructose	1-3 "
Galactose	14-20 "
Glucose	2-5 "
Lactose	50-60 "
Maltose	60 "
Melezitose	60 "
Raffinose	40-50 "
Sucrose	30 "

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TIME TABLE OF OSAZONE FORMATION

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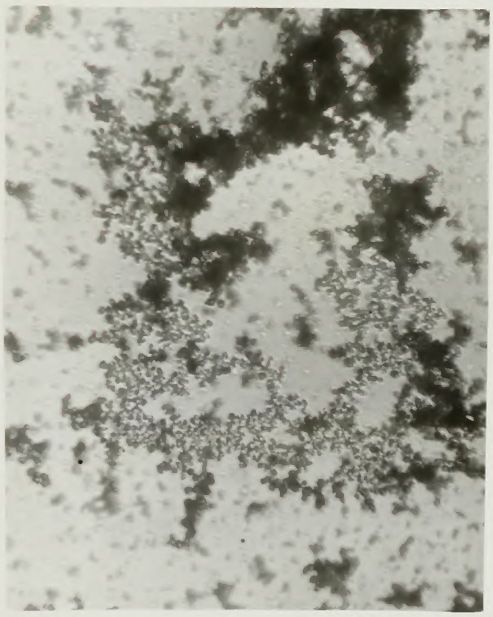
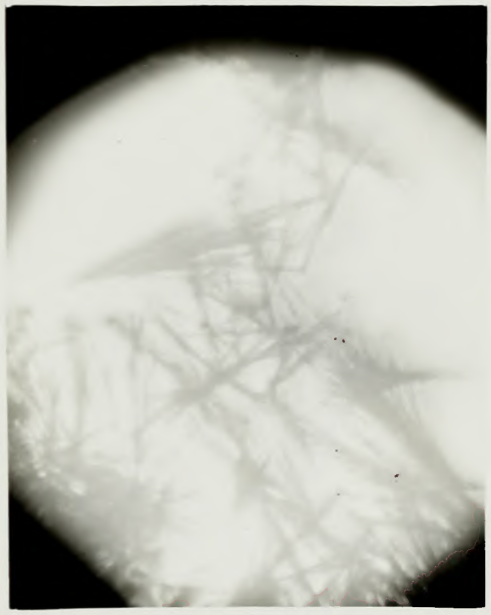
Galactone	14-20 "
Glucone	2-5 "
Lactone	30-60 "
Maltone	60 "
Melezitone	60 "
Raffinone	40-50 "
Sucrone	30 "



Arabinose (x 87)

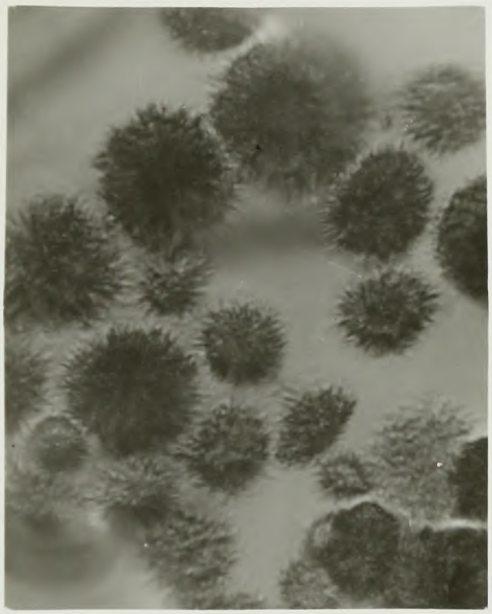


Fructose (x 87)

Fructose (x 87)

Galactose (x 87)

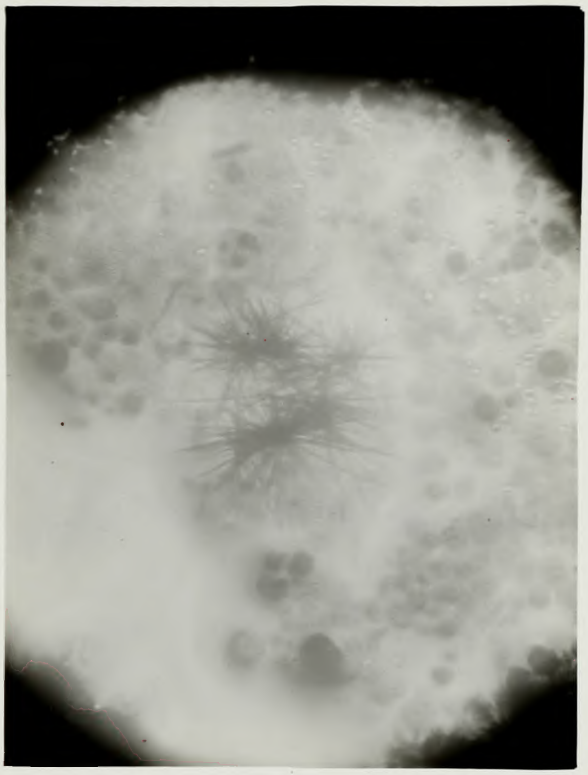
Glucose (x 87)



Lactose (x 385)

Maltose (x 500)

Melezitose (x 87)



Raffinose (x 87)

Sucrose (x 87)

QUANTITATIVE DETERMINATION OF GLUCOSE BY BENEDICT'S METHOD

Benedict's Reagent was prepared as follows: (a) 18 grams of hydrated copper sulfate weighed accurately were dissolved in 100 cc. of water. (b) 200 grams of hydrated sodium carbonate, 200 grams of hydrated sodium citrate and 125 grams of potassium thiocyanate were dissolved in about 600 cc. of hot water. The solution was filtered if necessary. Solution (a) was added to solution (b) slowly with constant stirring. Then 5 cc. of a 5% solution of potassium ferrocyanide were added. The solution was cooled and diluted to exactly one liter.

Principle: Reduction of a standard copper (cupric) sulfate solution (Benedict's Reagent) to the colorless cuprous state.

Procedure: One cc. of Benedict's Reagent was measured accurately by a pipette into a 6 inch test tube which was supported in a clamp on a ring stand. A small crystal of sodium carbonate (hydrated) was added to the solution. This was heated to boiling until the sodium carbonate dissolved.

A graduated 1 or 2 cc. pipette in a rubber stopper was supported in another clamp above the test tube. To the end of the pipette was attached a rubber tube with a pinch clamp and glass tubing. This is the burette. The unknown glucose solution was sucked into the pipette and the reading recorded. The tip of the pipette was then wiped dry so that any of the glucose solution which might adhere to the tubing

and fall into the Benedict's Reagent and cause an error in the work. The Benedict' Reagent in the test tube was kept boiling all the time. The glucose solution was dropped in a drop at a time until the solution was colorless. This is the end point and a white precipitate formed. Between the addition of each drop a full minute was allowed to elapse in order to permit the complete reduction to take place. If the mixture became concentrated during the process water was added to replace that lost by evaporation. When the endpoint was reached the reading was recorded.

Two determinations were made and if the volume of the glucose solution used both times was the same the average was taken and the calculation made. If they were different, a third determination was made.

Calculation: The reduction of 1 cc. of copper sulfate reagent is accomplished by exactly 2 mgm. of glucose. This weight of glucose is exactly contained in the number of cc. of glucose used in the reduction. From this data were calculated (a) the number of mgm. of glucose in 1 cc. of the unknown solution and (b) the percent of glucose in the unknown solution.

Readings:

2nd	0.39 cc.	0.77 cc.	1.14 cc.
1st	0.00	0.39	0.77
	0.39 cc.	0.38 cc.	0.37 cc.

$$0.38 : 0.002 :: 1 : x$$

$$x = 0.005262 \text{ mgm./cc.}$$

$$x = 0.52\%$$

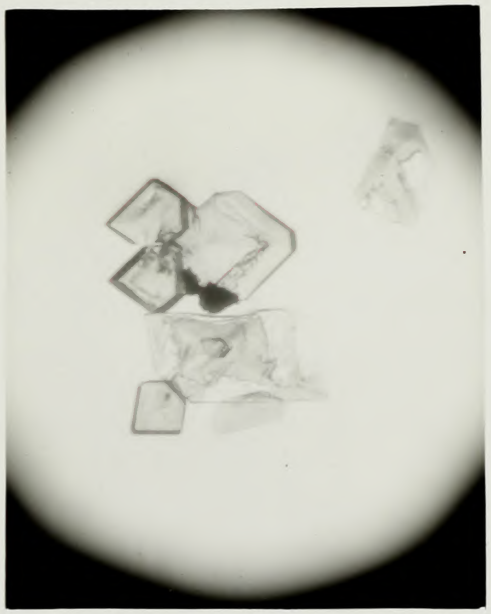
The unknown glucose solution used in this experiment was made up 0.525 grams per 100 cc. water.

UREA NITRATE

One drop of 10% urea solution and 1 drop of dilute nitric acid were placed on a slide. These were mixed and the crystals of urea nitrate formed very slowly taking about 10 minutes. They were viewed under the microscope and were very typical when compared with those on page 598 in Hawk and Bergeim's "Practical Physiological Chemistry." When concentrated nitric acid was used the crystals formed much more quickly than when dilute acid was used, but they were very small in size. However if the mixture was spread out over the slide, the individual crystals formed more rapidly and could be seen plainly. The crystals are in the form of monoclinic hexagonal or rhombohedral plates which often overlap like roofing tile. A microphotograph of these crystals is shown on the next page.

UREA NITRATE

One drop of 10% urea solution and 1 drop of dilute nitric acid were placed on a slide. These were mixed and the crystals of urea nitrate formed very slowly taking about 10 minutes. They were viewed under the microscope and were very typical when compared with those on page 398 in Hawk and Bergeim's "Practical Physiological Chemistry." When concentrated nitric acid was used the crystals formed much more quickly than when dilute acid was used, but they were very small in size. However if the mixture was spread out over the slide, the individual crystals formed more rapidly and could be seen plainly. The crystals are in the form of monoclinic hexagonal or rhombohedral plates which often overlap like roofing tile. A photomicrograph of these crystals is shown on the next page.



Urea Nitrate (x 87)

PREPARATION OF UREA FORMALDEHYDE RESIN

One tenth of a gram of urea was placed in a small beaker. To this, 3 drops of 37-40% formaldehyde solution were added. The mixture was stirred until all the urea had dissolved and then the mixture was allowed to stand. After a few minutes it became turbid and some heat was evolved. Then 2 drops of dilute HCl were added, the mixture stirred thoroughly; then a great amount of heat was evolved and the resin became very hard as it set.

This same reaction was carried out on a slide using a speck of urea (amount fitting on the tip of a spoon). To this 3 drops of HCHO were added and in 3 minutes turbidity appeared. Then 3 drops of dilute HCl were added, the resin began immediately to set or harden. The heat was not so easily observed in this method as in the beaker.

REACTIONS OF ACETOACETIC ESTER

1. Ferric chloride test. One drop of acetoacetic ester was added to 1 cc. of water in a micro test tube and the mixture shaken. To this mixture was added 1 drop of 10% ferric chloride solution which gave a deep purple color.

2. Formation of the copper salt. One drop of acetoacetic ester was added to 10 drops of ammoniacal copper sulfate solution in a micro test tube. The mixture was shaken and a turquoise blue precipitate was formed.

3. Sodium bisulfite test. Three drops of acetoacetic ester were added to 6 drops of saturated sodium bisulfite solution. These were shaken for 2 or 3 minutes and heat was given off.

4. Bromine water test. One drop of acetoacetic ester and 10 drops of bromine water were added to 10 drops of water in a micro test tube. The mixture was shaken and the solution became decolorized.

5. Potassium permanganate test. One drop of acetoacetic ester and 10 drops of 0.1% alkaline solution of potassium permanganate were added to 10 drops of water. The mixture was shaken and the solution became decolorized.

AMINO ACIDS AND PROTEINS

A. Buffer Action.

1. Ten drops of water were placed in one micro test tube and 10 drops of 1% glycine solution in another. One drop of Congo Red solution was added to each of these tubes. The color difference between these two solutions was not very marked; the water solution turned a bright orange and the glycine solution turned a darker orange. One drop of approximately 0.1 N HCl solution was added to each test tube. Both turned purple, but the water solution was darker. The HCl was added to the glycine solution drop by drop until it was the same shade as the water solution. About 10 drops were used.

2. The above experiment was repeated using phenolphthalein as indicator and adding an approximately 0.1 N NaOH solution instead of the HCl solution. Both solutions were colorless on the addition of the phenolphthalein, but on the addition of the 0.1 N NaOH solution the water solution turned to a deep pink and the glycine solution turned to a light pink. Seven more drops of NaOH were added to make the two solutions the same shade.

3. Both of the above experiments were repeated using 1% gelatin solution instead of the glycine solution. After the addition of the one drop of the 0.1 N HCl solution to both the water and the gelatin solution, 10 more drops were added to the gelatin solution to make it the same shade as the water solution. After the addition of the one drop of the 0.1 N NaOH

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solution to both the water and gelatin solution, 6 drops more were added to the gelatin solution to make it the same shade as the water solution.

These reactions of buffer action worked as well on a spot plate as in the test tube and were just as rapid. The colors were easily and clearly seen against the white background of the spot plate.

Since, in most of the following tests a half cubic centimeter of filtered egg albumin was used, it was found very efficient to place the egg albumin in a burette from which the half cubic centimeter could be measured accurately and quickly. The filtered egg albumin was of 1% strength.

B. Precipitation Reactions of Proteins.

1. Acids.

(a) A test tube containing 10 drops of concentrated nitric acid was held at an angle of 45 and ½ cc. of filtered egg albumin was allowed to flow slowly down the side of the tube. A white precipitate was formed at the junction of the two liquids. This is known as Heller's Ring Test for Proteins.

(b) Two drops of sulphosalicylic acid were added to ½ cc. of filtered egg albumin. A heavy white precipitate was formed.

2. Salts of Heavy Metals.

(a) Five drops of 10% mercuric chloride were added to ½ cc. of filtered egg albumin. A white precipitate was formed. Then 10 drops of saturated sodium chloride solution

were added the white precipitate dissolved. Then 3 drops of dilute HCl were added and the white precipitate reformed.

(b) Three drops of 10% lead acetate solution were added to ½ cc. of filtered egg albumin. A white precipitate was formed.

3. Acids of High Molecular Weight.

(a) Three drops of dilute HCl were added to ½ cc. of filtered egg albumin to make it slightly acid. Then 5 drops of phospho-molybdic acid were added and a heavy pale yellow precipitate was formed.

(b) Three drops of dilute HCl were added to ½ cc. of filtered egg albumin to make it slightly acid. Then 4 drops of tannic acid were added. A white precipitate was formed.

(c) Three drops of acetic acid were added to ½ cc. of filtered egg albumin. These were mixed by shaking and then 5 drops of 10% potassium ferrocyanide solution were added. A white precipitate was formed.

C. Color Reactions.

1. Biuret Reaction. One half cc. of filtered egg albumin was mixed with 1 cc. of 8% NaOH solution and 5 drops of 0.5% copper sulfate solution were added. The characteristic violet color was produced.

2. Millon's Reaction. Four drops of Millon's Reagent (solutions of mercuric and mercurous nitrates) were added to ½ cc. of filtered egg albumin. A white precipitate was formed which turned to brick red or a pink brown color when heated.

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3. Xanthoproteic Reaction. Four drops of concentrated nitric acid were added to ½ cc. of filtered egg albumin. A white precipitate was formed which turned to yellow on heating and to orange when it was made alkaline with ammonium hydroxide.

4. Hopkins-Cole Reaction. One half cc. of filtered egg albumin was mixed with an equal amount of Hopkins-Cole reagent (an aqueous solution of glyoxylic acid). Another test tube containing 1 cc. of concentrated sulfuric acid was held at an angle of 45 and the albumin-glyoxylic acid mixture was allowed to run slowly down the side of the tube into the sulfuric acid. A deep purple color was produced at the junction of the two liquids. Sometines a slight shake was necessary to produce the color.

D. Iso-electric Point of Proteins.

Acetic acid was added a few drops at a time to 10 drops of 1% alkaline solution of casein until no further change occured. A precipitate was formed at first which dissolved on the addition of more acetic acid because only at the iso-electric point do proteins have the least tendency to stay in solution and so become precipitated.

3. Xanthoproteic Reaction. Four drops of concentrated nitric acid were added to 4 cc. of filtered egg albumin. A white precipitate was formed which turned to yellow on heating and to orange when it was made alkaline with ammonium hydroxide.

4. Hopkins-Cole Reaction. One half cc. of filtered egg albumin was mixed with an equal amount of Hopkins-Cole reagent (an aqueous solution of glyoxylic acid). Another test tube containing 1 cc. of concentrated sulfuric acid was held at an angle of 45° and the albumin-glyoxylic acid mixture was allowed to run slowly down the side of the tube into the sulfuric acid. A deep purple color was produced at the junction of the two liquids. Sometimes a slight shake was necessary to produce the color.

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FATS, FATTY ACIDS AND GLYCEROL

A. FATS.

1. The testing the solubility of cottonseed oil in water, ethanol, chloroform, ether and carbon tetrachloride was performed by using 4 drops of the oil and ½ cc. of the solvent in each case. The oil is insoluble in water, forms an emulsion with ethanol which settles out on standing, and is soluble in ether, chloroform and carbon tetrachloride.

2. A drop of oil was placed on a piece of paper and a transparent spot was formed. This is called the "transparent spot" test.

3. Cottonseed oil was tested with litmus, phenolphthalein and Congo Red using 8 drops of oil and 1 drop of indicator. This was done both in a micro test tube and on a white spot plate. No change was observed.

These tests were repeated using 1 drop of rancid olive oil made by adding 1 drop of oleic acid to 2 cc. of olive oil instead of the cottonseed oil. The Congo Red solution was the only one that changed, this turning a blue-brown, in 5 minutes in the test tube reaction and in 15 minutes on the spot plate.

4. Acrolein Test. On a watch glass were ground together 5 drops of cottonseed oil and the same amount of dry potassium acid sulfate. The mass was transfered to a test tube and was heated cautiously. The irritating odor of acrylic aldehyde was detected which is a positive test.

5. One drop of cottonseed oil was added to 1 cc. of water in a micro test tube. The mixture was shaken thoroughly forming an emulsion which is not permanent. One drop of a 1% solution of sodium carbonate was added to the mixture and a permanent emulsion was obtained.

A mixture of 1 drop of cottonseed oil, 1 drop of 1% soap solution and 1 cc. of water were shaken together in a micro test tube. The emulsion seemed to be even more stable than the one formed above.

6. A small speck of lard was dissolved in 2 drops of ether in a test tube. Two drops of alcohol were added and the mixture was allowed to evaporate spontaneously. Crystals should form but even by varying the amounts and the methods used, no crystals could be obtained and each time the fat globules settled out. These lard crystals were to be compared with those found on page 251 in Hawk and Bergeim's "Practical Physiological Chemistry." The class results of this on a macro scale were not good.

7. One gram of potassium hydroxide was dissolved in 10 cc. of water in a small casserole and 2 grams of bayberry tallow were added to this. The mixture was boiled and the amount of water was kept constant by adding some from time to time. Saponification was complete when no oil settled out when 1 drop of the mixture was placed in a micro test tube of water. Saponification took place in about 5 minutes. The mixture was allowed to cool. Then 5 cc. of it were transfered

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7. One gram of potassium hydroxide was dissolved in 10 cc. of water in a small casserole and 2 grams of babberry tallow were added to this. The mixture was boiled and the amount of water was kept constant by adding some from time to time. Saponification was complete when no oil settled out when 1 drop of the mixture was placed in a micro test tube of water. Saponification took place in about 5 minutes. The mixture was allowed to cool. Then 5 cc. of it were transfered

to a small beaker and saved for B 6.

The remainder was acidified by adding concentrated HCl slowly and with stirring. The solution was cooled and the palmitic acid which rose to the top was removed. This palmitic acid was washed twice with water by decantation. It was then transfered to a 50 cc. flask and dissolved in 5 cc. of alcohol and heated on a water bath. A small reflux condenser consisting of a long piece of glass tubing was attached to the flask before heating. When the acid had dissolved, the solution was filtered, and the filtrate allowed to cool slowly. Pale yellow crystals of palmitic acid settled out. The solution was filtered by suction.

B. Fatty Acids.

1. The crystals of palmitic acid were examined under the microscope by dissolving a little of the solid in a drop of alcohol on a slide. The crystals were compared with those found on page 252 in Hawk and Bergeim's "Practical Physiological Chemistry." Those obtained by this method were very small but were characteristic.

2. The testing of the solubility of palmitic acid in water, ethanol, chloroform, ether and carbon tetrachloride was performed by using a speck of the solid and ½ cc. of the solvent in each case. The acid was insoluble in water, but soluble in the other solvents.

3. A speck of palmitic acid was melted and then dropped on a piece of paper. A positive transparent spot test was ob-

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3. A speck of palmitic acid was melted and then dropped on a piece of paper. A positive translucent spot test was ob-

tained.

4. The acrolein test was applied to palmitic acid. A speck of the acid was ground intimately with a speck of dry acid potassium sulfate. The mixture was transfered to a dry test tube and heated. The irritating odor of acrylic aldehyde was not detected and thus the test was negative.

5. A small amount of the palmitic acid was dissolved in ½ cc. of chloroform. To this 2 drops of Hübl's solution (made by dissolving 26 grams of iodine and 30 grams of mercuric chloride in 1 liter of 95% ethanol) were added. The mixture was shaken and a clear red solution was obtained.

The reaction was repeated using only the chloroform and Hübl's solution. A clear brown solution resulted.

The reaction was again repeated using oleic acid instead of palmitic acid. A clear yellow solution resulted showing that the oleic acid was unsaturated and for this reason decolorized the Hübl's solution.

6. To the 5 cc. alkaline solution saved from A7, solid sodium chloride was added with constant stirring until the solution was saturated. The soap curd that formed was skimmed off. A little of the curd was dissolved in about 10 drops of water. This solution was divided into two parts. To one 5 drops of 10% calcium chloride solution were added and a flaky white precipitate was formed. To the other 5 drops were added 5 drops of 10% magnesium sulfate solution and a fine yellow precipitate was formed.

C. Glycerol.

1. The testing of the solubility of glycerol in water, alcohol and ether was performed by using 4 drops of glycerol and 10 drops of solvent. The glycerol was soluble in water and alcohol but insoluble in ether.

2. A positive transparent spot test was obtained by dropping 1 drop of glycerol on paper.

3. Cupric hydroxide was formed by adding 1 cc. of 10% copper sulfate solution to ½ cc. of 10% sodium hydroxide solution. The precipitate was washed by decantation by using water, or was filtered and then washed with water. A little of the precipitate was suspended in 1 cc. of water and 10 drops of glycerol were added. The mixture was shaken and the precipitate remained in suspension.

4. The acrolein test was applied to glycerol with positive results. On a watch glass were ground together 5 drops of glycerol and the same amount of dry potassium acid sulfate. The mass was transfered to a micro test tube and heated. The irritating odor of acrylic aldehyde was obtained.

PROPERTIES OF BENZENE

1. Two drops of benzene were placed on a glass slide and the odor was observed to be like that of gasoline.

2. Ten drops of water were added to 2 drops of benzene. Benzene was not soluble in water and its specific gravity is less than one.

3. A drop of benzene was placed on a microscope slide and a lighted match was brought near the benzene. The benzene burst into flame and burned with a bright yellow and a very smoky flame.

4. One drop of benzene was mixed with 1 drop of alkaline potassium permanganate solution (0.1% containing 5 grams of NaOH per liter). A green solution resulted, which on heating turned to a brownish purple. This can be done on a slide or in a test tube.

5. One drop of 2% solution of bromine in carbon tetrachloride was added to 10 drops of benzene in a micro test tube. The mixture was shaken and the color of the solution turned from a red orange to a yellow. The test tube was corked and allowed to stand. In fifteen minutes the mixture had been decolorized. A control was used of 1 drop of 2% solution of bromine in carbon tetrachloride added to 10 drops of carbon tetrachloride. The color of the control remained the same during the time of the experiment, therefore there was a definite color change in the reaction mixture of benzene and bromine in carbon tetrachloride.

6. Five drops of benzene were placed in a micro test tube and to this 1 drop of bromine (not in solution) was added. To this mixture several pieces of clean iron filings were added. The mixture was shaken well. White fumes came out of the test tubes. After the reaction was over the mixture was poured into a beaker of water. Dense white fumes came off and a white oil, bromobenzene, remained in the bottom of the beaker. Therefore the specific gravity of bromobenzene was greater than one.

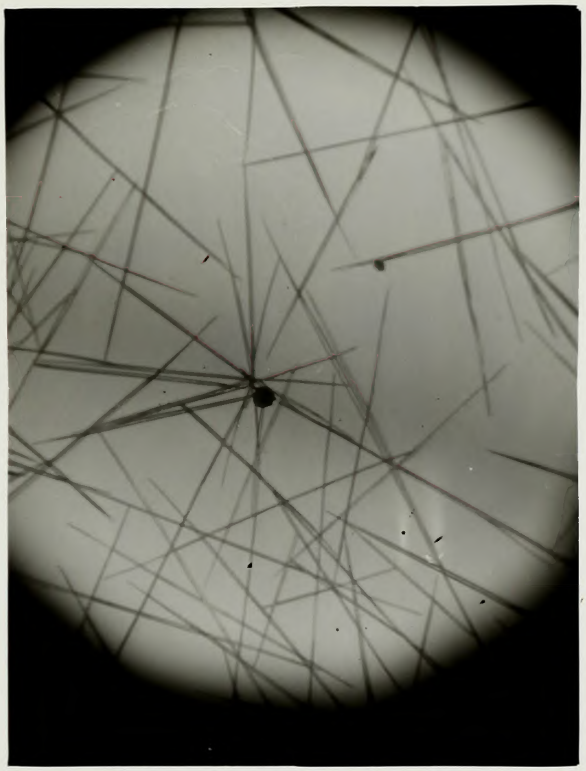
7. In a micro test tube 10 drops of concentrated sulfuric acid and 4 drops of concentrated nitric acid were mixed by shaking. To this mixture 4 drops of benzene were added drop by drop and the mixture shaken constantly during the addition. When the reaction was complete, as was seen by the disappearance of the benzene, the whole mixture was poured into a beaker of water. The product, nitrobenzene, was a heavy, insoluble oil which smelled sweet like phenol.

6 PREPARATION OF DINITROBENZENE

In a 4 inch test tube 1 cc. of concentrated nitric acid was mixed with 2 cc. of concentrated sulfuric acid. To this 4 drops or 0.2 cc. of benzene were added a drop at a time. The tube was shaken during the addition and then heated gently for 5 minutes. The solution was cooled and then poured into 10 cc. of water. The dinitrobenzene settled out as a yellow solid. The solution was filtered by suction and the precipitate washed with water until the filtrate was fairly clear. The precipitate was dissolved in dilute ethanol (1:1) by warming gently. The solution was filtered hot and allowed to cool. The crystals formed slowly. The solution was filtered by suction and the residue dried. Some of the crystals were dissolved in ethanol and observed under the microscope. There they appeared as long white needles.

When the residue was thoroughly dry, a melting point was taken and was found to be 90 C.

A micro photograph of these dinitrobenzene crystals under low power is shown on the next page.



Dinitrobenzene (x 87)

PREPARATION OF PHENOL

From the sodium salt of benzenesulfonic acid. One pellet of sodium hydroxide was melted in a porcelain crucible. A speck of sodium benzene sulfonate was added to this. The mixture was heated 2 minutes. Care was taken not to heat the mixture too strongly to avoid charring. The mass was allowed to cool. It was then dissolved in 1 cc. of water and acidified with about 1 cc. of hydrochloric acid. The mixture was filtered. The odor of phenol was detected in the filtrate. The filtrate was saved for the experiment on the properties of phenol.

PROPERTIES OF PHENOL

1. In a micro test tube 5 drops of the phenol filtrate saved from the experiment on the preparation of phenol were mixed with 5 drops of bromine water. A yellow-orange precipitate of 2-4-6 tribromophenol was formed. Five drops of the 2% solution of phenol may be used as well as the filtrate mentioned above in case the experiment on the preparation of phenol was not performed.

2. One drop of 1% solution of ferric chloride was added to 5 drops of 2% solution of phenol in a micro test tube. A purple color was produced which is characteristic of phenols.

3. Making of phthalein colors. See experiment on phthalein colors.

PROPERTIES OF ANILINE

1. Solubility. The testing of the solubility of aniline in water, ethanol and benzene was performed by using 1 drop of aniline and 5 drops of the solvent. This may be done either in a test tube or on a microscope slide although the test tube method is better. Aniline is soluble in ethanol and benzene and insoluble in water.

2. With bromine. Six to 8 drops of bromine water were added a drop at a time to an aqueous solution of aniline (made by adding 1 drop of aniline to 3 drops of water). The solution became yellow and tribromaniline was precipitated.

3. With nitrous acid. One drop of aniline was dissolved in 2 drops of concentrated hydrochloric acid and 5 drops of water. The solution was cooled with running water if it was necessary. Five drops of a cold solution of sodium nitrite (10%) were added. The mixture was shaken and then heated gently. A gas, nitrogen, was given off and the odor of the solution was that of phenol. The color of the solution was orange and a brown oil formed the surface layer.

4. With ferric chloride. Five drops of 5% ferric chloride solution were added to an aqueous solution of aniline. A red brown precipitate of ferric hydroxide was formed and the aniline hydrochloride was in solution.

5. With hypochlorites. Five drops of a clear solution of bleaching powder or of sodium hypochlorite were added to an aqueous solution of aniline. A red purple solution re-

sulted.

6. With potassium dichromate. In a 30 cc. beaker 1 drop of aniline was mixed with 3 drops of concentrated sulfuric acid and 2 drops of 10% potassium dichromate solution. A blue color was formed. The mixture was heated for a few minutes on a water bath (made by inserting the 30 cc. beaker into a 50 cc. beaker containing hot water). A blue solid resulted.

7. Aniline hydrochloride. In a small beaker 10 drops of aniline, 10 drops of water and 20 drops or 1 cc. of concentrated HCl were heated for 10 minutes on a water bath. The mixture must not be heated to strongly or all the liquid will be boiled off. The solution was allowed to cool by being placed in a beaker of cold water. Crystals of aniline hydrochloride settled out. These were filtered off by suction and washed with ether and allowed to dry. The solid was used for the following reactions.

8. Hydrolysis of aniline salts. A speck of the aniline hydrochloride was dissolved in a drop of water on a slide. The solution was tested with blue litmus which turned red, therefore the solution was acidic.

9. Reaction of aniline salts with bases. A speck of the aniline hydrochloride was dissolved in 3 drops of water, either in a test tube or on a slide. To this 3 drops of 10% sodium hydroxide solution were added. An emulsion resulted. This reaction was also carried out using one drop of each reagent but only a slight turbidity resulted.

PREPARATION OF PHTHALEIN COLORS

Phenolphthalein. A speck of solid phenol about equal to ½ gram was placed in a micro test tube. To this about double its volume of phthalic anhydride was added. To this mixture 5-6 drops of concentrated sulfuric acid were added. The mixture was heated about 3 minutes in an oil bath to 180 C. The mass was cooled, dissolved in 10 drops of water and made alkaline with 10 drops of 10% sodium hydroxide solution. The cerise color formed was due to the phenolphthalein.

Fluorescein. A speck of solid resorcinol about equal to ½ gram was placed in a micro test tube. About double its volume of phthalic anhydride was added to this. Five or 6 drops of concentrated sulfuric acid were added to the mixture. The entire mixture was heated in an oil bath for 3 minutes to about 180 C. The mass was cooled, and dissolved in 10 drops of 10% sodium hydroxide solution. Fluorescence was noted. The color of the solution was an orange green by reflected light and red by transmitted light.

Eosin. The fusion above was repeated. To the solid mass 5 drops of glacial acetic acid and 1 drop of bromine were added, the mixture was warmed gently. The mass was cooled, diluted with 10 drops of water and made alkaline with 10 drops of 10% sodium hydroxide solution. The color of the solution was a deep green by reflected light and a deep red by transmitted light.

CONCLUSION

The micro method in organic chemistry does save time, materials and space. It does develop an additional technique and accuracy in the student.

The micro method was successfully adapted to the washing and settling of precipitates, filtration, and to a certain extent, distillation. However this laboratory process on the micro scale can be improved. The microscope and centrifuge were used to advantage in performing some of the experiments. Test tube reactions and color tests worked especially well when done by this method.

Micro organic chemistry is still in its infancy but has great possibilities for further development.

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